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DATA EVALUATION RECORD

STUDY TYPE: *In Vitro* Dermal Penetration Test
OPPTS 870.7600

DP BARCODE: D261221
P.C. CODE: 111801
SUBMISSION CODE: S569727
CASE TYPE: Reregistration

TEST MATERIAL (PURITY):
PHMB (20.2%) and [^{14}C]-PHMB (Specific activity:185GBq/732mg, the purity of radio-labeled test substances has not been reported)

CITATION: Clowes, H.M. 1996. PHMB: In Vitro Absorption Through Human Epidermis. September 6, 1996. Report No. CTL/P/5120. Prepared by Zeneca Central Toxicology Laboratory, Cheshire, UK. [MRID 44119301].Unpublished Report.

SPONSOR: Zeneca Inc.
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Wilmington, DE 19850-5457

EXECUTIVE SUMMARY:

To evaluate the potential of penetration of PHMB through human skin, the absorption of PHMB was measured *in vitro* through human epidermis. A flow-through skin on chamber design was used and three aqueous PHMB concentrations (nominal concentrations of 200g /l, 20g/l and 2g/l) were applied. These dilutions were applied to the epidermal membranes at a rate of 10µl/cm^2; the 200g/l concentration was also applied at 200µl /cm^2. The 10µl/cm^2 applications were left unoccluded throughout the entire exposure period and the 200 µl/cm^2 was occluded.

The *in vitro* results indicated the absorption of PHMB from the nominal 20% solution through human epidermis was slow and linear over the 96h exposure period. The permeability coefficient (Kp) has been calculated to be 5.47 x 10^{-7} cm/h from the steady state rate of absorption (0-24h). Approximately 13 times more was absorbed from the 200µl/cm^2 occluded application (0.088µg/cm^2 /h) than from the 10µl/cm^2 unoccluded application (0.007µg/cm^2 /h).

The absorption rate of PHMB was very slow from the nominal 2% solution (0.005µg/cm^2 /h) during the first 24h and slowed down further between 24 and 96h (0.003µg/cm^2 /h). Absorption from the nominal 0.2% solution was measured at 0.001µg/cm^2 /h during the first 24h and was below the limit of quantitation over the 24-96h period.

This study indicates a slow rate and extent of absorption of PHMB through human epidermis. However, this system did not take into account other skin structures such as dermis which may have an influence on PHMB absorption. In addition, these data do not account for the influence of systemic distribution and excretion kinetics upon dermal absorption of PHMB. Thus, this study is classified as **unacceptable** and does not satisfy the OPPTS guideline for dermal penetration study (OPPTS 870.7600).

COMPLIANCE: Signed and dated Quality Assurance, Good Laboratory Practice, Data Confidentiality, and Flagging statements were included.

I. MATERIALS AND METHODS

A. TEST SUBSTANCES

A.1 PHMB

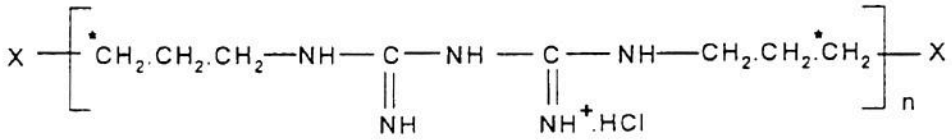
Chemical name:	Polyhexamethylene biguanide (PHMB)
PHMB content:	20.2%
Source:	Zeneca Inc. Dighton, Mass., USA
Color:	Faint Yellow
Physical state:	Liquid
Storage conditions:	Ambient temperature and in the dark

A.2 [^{14}C] - PHMB

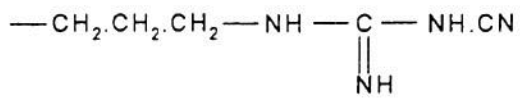
Source: Cambridge Research Biochemicals, Northwich, Cheshire, UK
Physical state: Faint yellow liquid
Radiochemical reference number: 4602
Specific activity: 1.85GBq/732mc,
Storage conditions: Freezer

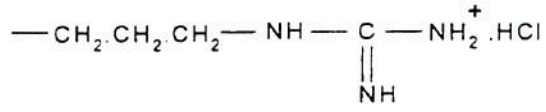
* denotes the position of ^{14}C-labelled atoms

n= 1-40



Where X = $-CH_2.CH_2.CH_2-NH_2^+.HCl$

or 

or 

A.2.1 Structure of the radiolabelled test substance

B. EXPERIMENTAL PROCEDURES

B.1 Liquid scintillation counting (LSC) conditions

Sample volume: 250µl
Counting period: 4 minutes or until 40000 counts had been registered
Scintillation fluid: Optiphase 'HISAFE' (10ml)
The limit of quantitation using the above procedure was set at between 0.002 and 0.2µg/ml depending on the application.

B.2 Preparation of epidermal membranes

Extraneous tissue was removed from human whole skin samples. No information was given regarding which part of the body where the skin was taken from. The skin samples were immersed in water at 60^{O}C for 40-45 seconds and the epidermis teased off the dermis. Each epidermal membrane was given an identifying number and stored frozen on aluminium foil until required for use.

B.3 Measurement of membrane integrity

Samples of epidermis were mounted in glass diffusion cells with an exposed area of 2.54cm^2. The cells were placed in a water bath maintained at 30 ± 1^{O}C. The integrity of the membranes was determined by measurement of their electrical resistance across the skin membrane. Membranes with a calculated resistance smaller than 10 k□ were regarded as being lower than normal and not used for exposure to the test material.

B.4 Measurement of test substance absorption

A flow-through skin on chamber design was used (See **Figure 1**). The receptor chambers of the cells were filled with a recorded volume of receptor fluid (distilled water) and placed in a water bath maintained at 30 ± 1^{O}C. A pre-treatment sample (0.25ml) was taken from each receptor chamber for analysis by LSC. An equal volume of fresh receptor fluid was added to each receptor chamber to replace the volume removed.

PHMB was applied to the skin membranes as dilutions in water at the dose rates indicated in **Table 1**. PHMB solutions were prepared by mixing cold PHMB with [^{14}C]-PHMB and distilled water to give the required concentrations. The [^{14}C]- PHMB was added in amounts to give activities ranging from approximately 3 x 10^6 dpm/ml for the 0.2% dose to approximately 4 x 10^9 dpm/ml for the 20% dose to maximize the analytical sensitivity of the varying dosing regimes. The 10µl/cm^2 applications were left unoccluded and the 200µl/cm^2 occluded (20% solution) for the duration of the exposure period (96h). These application doses were designed to simulate potential dermal exposure to PHMB during normal use.

At recorded intervals, samples (0.25ml) of the receptor fluid were taken for analysis by LSC. The volume of fluid in the receptor chamber was maintained by the addition of (0.25ml) of fresh receptor fluid to the chamber immediately after the removal of each sample.

II. RESULTS AND DISCUSSION

The results obtained in this study are summarized in **Tables 1 and 2**, where data are presented both in terms of absorption rate and in terms of amount and percent of the dose applied at 6, 8, 24 and 96h after exposure.

Absorption of PHMB from the nominal 20% solution through human epidermis was slow and linear over the 96h exposure period. The permeability coefficient (Kp) has been calculated to be 5.47 x 10^{-7} cm/h from the steady state rate of absorption (0-24h). Approximately 13 times more was absorbed from the 200µl/cm^2 occluded application (0.088µg/cm^2 /h) than from the 10µl/cm^2 unoccluded application (0.007µg/cm^2 /h).

The absorption rate of PHMB was very slow from the nominal 2% solution (0.005µg/cm^2 /h) during the first 24h and slowed down further between 24 and 96h (0.003µg/cm^2 /h). Absorption from the nominal 0.2% solution was measured at 0.001µg/cm^2 /h during the first 24h and was below the limit of quantitation over the 24-96h period.

For the 20, 2 and 0.2% solutions from the 10µl/cm^2 unoccluded applications, absorption was slow and linear over the initial 24 hours. The steady state rates of absorption are tabulated below:

III. SUMMARY AND CONCLUSION

This study indicates a slow rate and extent of absorption of PHMB through human epidermis. However, this system did not take into account other skin structures such as dermis which may have an influence on PHMB absorption. In addition, these data do not account for the influence of systemic distribution and excretion kinetics upon dermal absorption of PHMB. Thus, this study is classified as **unacceptable** and does not satisfy the OPPTS guideline for dermal penetration study (OPPTS 870.7600).

TABLE 1. MEAN PHMB ABSORPTION RATE THROUGH HUMAN EPIDERMIS [a]						
Concentration (g/L)	**Actual Concentration (g/L)**	**Applied Volume (µL/cm²)**	**Applied Dose (µg/cm²)**	**Number of Samples**	**Time Period (hour)**	**Absorption Rate (µg/cm²/hour ± SEM)**
2	1.93	10[b]	20	8	0 - 24	0.001 ± <0.001
					24 - 96	<0.001 ± <0.001
					0 - 96	<0.001 ± <0.001
20	20.9	10[b]	200	8	0 - 24	0.005 ± <0.044
					24 - 96	0.003 ± <0.001
					0 - 96	0.003 ± <0.001
200	197	10[b]	2000	5	0 - 24	0.009 ± 0.003
					24 - 96	0.007 ± 0.002
					0 - 96	0.007 ± 0.002
200	201	200[b]	402000	4	0 - 24	0.110 ± 0.044
					24 - 96	0.083 ± 0.029
					0 - 96	0.088 ± 0.033

Note: a. Taken From Page 19 Table 1.

The 10µl/cm² applications were unoccluded throughout the entire period and the 200 µl/cm² was occluded. .

TABLE 2. MEAN PHMB ABSORBED THROUGH HUMAN EPIDERMIS [a]							
Concentration (g/L)	**Actual Concentration (g/L)**	**Applied Volume (µL/cm²)**	**Applied Dose (µg/cm²)**	**Number of Samples**	**Exposure Time (hours)**	**Amount Absorbed (µg/cm²)**	**Percent Absorbed (%)**
2	1.93	10[b]	20	8	6	0.007	0.036
					8	0.008	0.041
					24	0.017	0.088
					96	0.025	0.129
20	20.9	10[b]	200	8	6	0.032	0.015
					8	0.043	0.020
					24	0.125	0.059
					96	0.307	0.146
200	197	10[b]	2000	5	6	0.123	0.006
					8	0.141	0.007
					24	0.237	0.012
					96	0.708	0.036
200	201	200[b]	402000	4	6	0.784	0.001
					8	1.04	0.002
					24	2.64	0.006
					96	8.47	0.021

Note: a. Taken From Page 19 Table 1.

b. The 10µl/cm² applications were unoccluded throughout the entire period and the 200 µl/cm² was occluded.

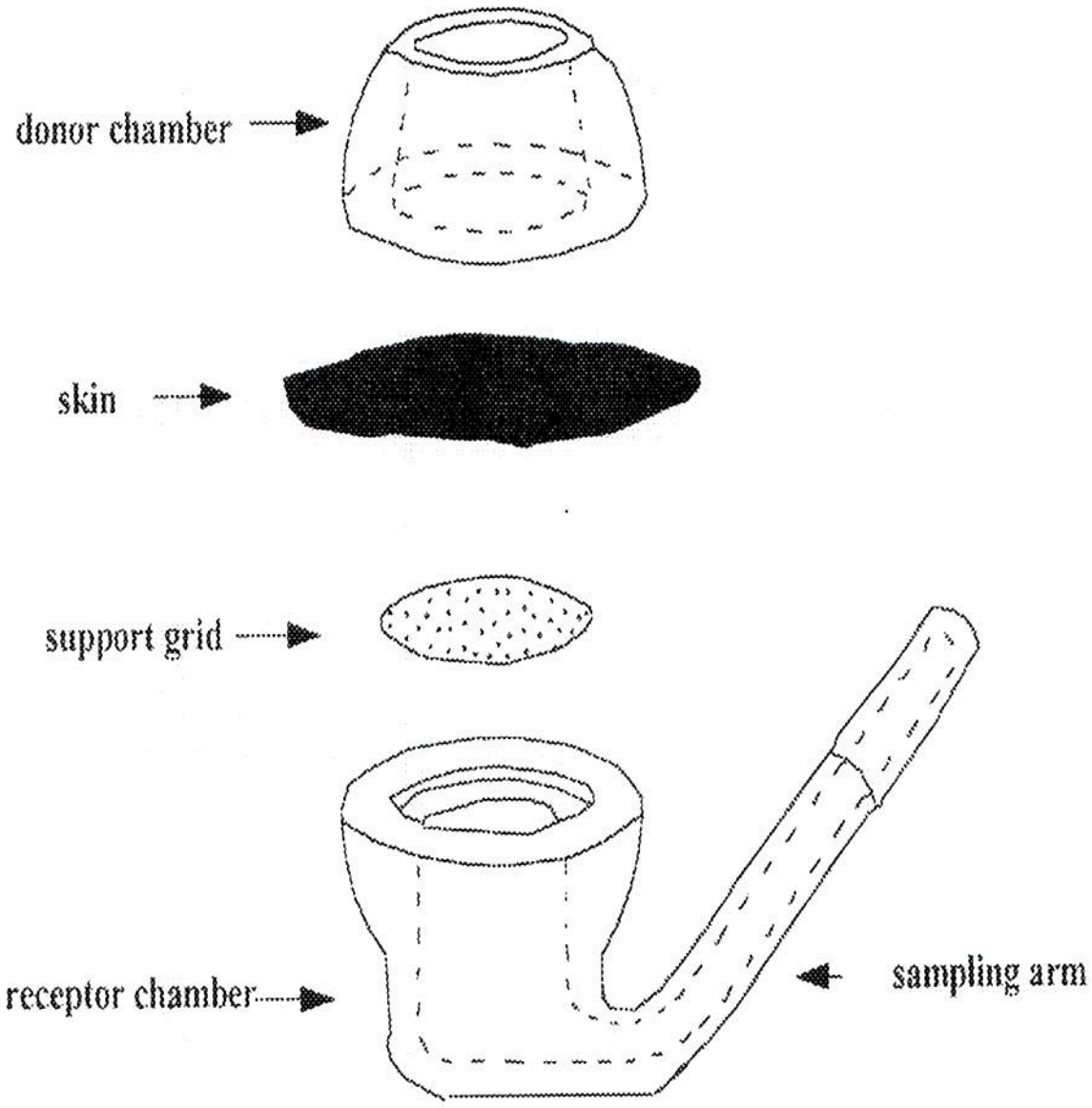


Figure 1. Diffusion Cell